

300 Rec'd PCT/PTO 19 JAN 1999

C TITLE OF THE INVENTION

Viral Interleukin-6

Sub C17
The present invention relates to viral interleukin-6 (v-IL-6), which can be obtained by recombinant expression of the DNA of human herpesvirus type 8 (HHV-8), and which may be used in diagnosis and treatment of human diseases such as kaposi sarcoma, Castleman's disease, multiple myeloma, kidney cell carcinoma, mesangial proliferative glomerulonephritis or B cell lymphoma.

09230048-031299
Kaposi's sarcoma (KS), a multifocal proliferative lesion of uncertain pathogenesis, is highly prevalent among homosexual AIDS patients. Studies with biopsy materials and cultured cells have indicated an important role of growth factors and cellular cytokines, such as basic fibroblast growth factor, interleukin-1 β , platelet derived growth factor, interleukin-6 (IL-6), and oncostatin M for the proliferation of spindle cells in KS^{1,2}. Several groups found indication for the expression of interleukin-6 (IL-6) receptors in AIDS-KS cells³ and derived spindle cell lines⁴. As epidemiological evidence had suggested that an infectious agent other than HIV may also be involved in KS pathogenesis, it stirred considerable interest when Chang and colleagues⁵ found DNA sequences of a novel herpesvirus in AIDS-KS tissues. Meanwhile, DNA of this virus was consistently found in all epidemiological forms of KS. The new virus, termed human herpesvirus 8 (HHV-8), shows marked sequence homology to *herpesvirus (h.) saimiri*, the prototype of γ_2 -herpesviruses; thus HHV-8 appears to be the first human

member of γ_2 -herpesviruses (genus rhadinovirus). Cloning HHV-8 DNA from KS tissues and sequencing indicates a genome organization that is generally collinear to *h. saimiri*⁶.

CS7
In the course of these studies we surprisingly found, adjacent to a dihydrofolate reductase gene, an open reading frame (ORF) with the coding capacity for a 204 amino acid polypeptide with marked homology to mammalian IL-6 (P-value for homology searches with NCBI-BLAST: $P \leq 10^{-18}$; percent identity/similarity to human IL-6: 24.74%/ 46.91%; to murine: 24.23%/ 47.94%; to porcine: 25.97%/ 52.91%; to bovine: 24.60%/ 49.73%; all alignments were calculated with the GCG software "GAP").

The viral gene product (v-IL-6) has conserved all 4 cysteine residues that are known to be involved in IL-6 disulfide bridging, and it shows a characteristic signal peptide of 19 to 22 amino acids (fig. 1). The area involved in binding of human IL-6 to its receptor has been mapped to the middle of the protein by two groups^{7, 8, 9}. Ehlers et al. showed that amino acids 105 to 123 of the human IL-6, as shown in fig. 1 (GFNEETCLVKLIIGLLEFE), are involved in receptor binding. *(residues 105-123 of STD ID No: 3)* Most remarkably, this region is highly conserved in v-IL-6 (GFNETsCLkKLadGFFEFE). *(residues 84-105 of STD ID No: 2)* Identity and similarity of v-IL-6 to the receptor binding region of human IL-6 are 58% and 74%, respectively (fig. 1). This is almost identical with the degree of conservation that can be observed in this receptor binding area of human IL-6 to murine IL-6. As both human IL-6 and murine IL-6 are able to bind to the receptor of the other species (murine IL-6 and human IL-6, respectively), it is likely that v-IL-6 is also able to bind to the human and the murine IL-6 receptor.

Rhadinoviruses frequently acquire genes from their host cell¹⁰. This HHV-8 ORF however, is the first known example of a viral IL-6 structural homologue. Up to now all cell-homologous genes of rhadinoviruses that have been tested were functional; non-functional genes would most likely have been lost in viral evolution. Thus, the conservation of essential IL-6 features makes it highly suggestive that v-IL-6 is

"SECRETED" 34000000

- 3 -

functional in normal HHV-8 replication or persistence. Since models of paracrine growth stimulation of spindle cells by cytokines, including IL-6 and the related oncostatin M, have been proposed for KS pathogenesis, the finding of the v-IL-6 gene in HHV-8 lends support to the hypothesis that HHV-8 is causally related to this multifocal proliferation.

The present invention therefore relates to:

- 662150-034003260
- B
- B
- a) Viral interleukin-6 (v-IL-6), which can be obtained by recombinant expression of the DNA of HHV-8.
- b) A polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2.
- c) A fragment of v-IL-6, having the capability of binding to an IL-6 receptor and comprising the amino acid sequence ^(residues 87-105 of SEQ ID NO:2) GFNEtsCLkKLadGFFEFE.
- d) A fragment as defined in b1, which essentially comprises the amino acid sequence ^(residues 87-105 of SEQ ID NO:2) GFNEtsCLkKLadGFFEFE.
- e) A fragment as defined in c or d, which binds to a human IL-6 receptor.
- f) A polypeptide having the amino acid sequence displayed in fig. 2.
- g) Mutants and variants of v-IL-6 or of the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2, which mutants and variants are obtained by conventional amino acid substitutions or deletions, with the proviso that these mutants and variants are functionally equivalent to v-IL-6.

- h) Fragments of v-IL-6, or of the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2, characterized in that they are able to competitively inhibit the biological activity of IL-6 in a suitable assay system.
- i) An isolated nucleic acid coding for v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2. A preferred embodiment is the nucleic acid having the nucleotide sequence of fig.2. Furthermore, an isolated nucleic acid, hybridizing to the abovementioned nucleic acids under stringent conditions and encoding functionally active v-IL-6 shall be comprised.
- k) Monoclonal or polyclonal antibodies directed against v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2.
- l) Testkit for the detection of v-IL-6 in a sample, comprising one or more of the above monoclonal or polyclonal antibodies.
- m) Testkit for the detection of antibodies against v-IL-6 comprising v-IL-6 and/or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2, and/or mutants and variants of v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2 and/or fragments of v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2.
- n) Testkit for the detection of v-IL-6 DNA or RNA, comprising a nucleic acid which codes for v-IL-6, or which hybridizes to the aforementioned nucleic acid and encodes functionally active v-IL-6.

- o) A medicament comprising as an active ingredient a monoclonal antibody or polyclonal antibodies directed against v-IL-6, or a polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2, or mutants, variants or fragments of v-IL-6 or the aforementioned polypeptide. In another embodiment, the medicament may comprise as an active ingredient a nucleic acid encoding v-IL-6.
- p) A cell culture growth medium, comprising as an active ingredient v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2, or mutants, variants or fragments of v-IL-6 or the aforementioned polypeptide.
- q) A process of manufacturing v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2, or mutants and variants, or fragments of v-IL-6 or the aforementioned polypeptide.
- r) A process of manufacturing a medicament, wherein the active ingredient is combined with suitable excipients and/or other auxiliary compounds according to common knowledge of those skilled in the art.
- s) A process of manufacturing a medicament comprising as an active ingredient monoclonal or polyclonal antibodies directed against v-IL-6, or a polypeptide comprising v-IL-6, or mutants, variants or fragments of v-IL-6, or a nucleic acid encoding v-IL-6 for the treatment of kaposi sarcoma, Castleman's disease, multiple myeloma, kidney cell carcinoma, mesangial proliferative glomerulonephritis or B cell lymphoma.

- t) A process of diagnosing an HHV-8 infection comprising the in vitro detection of v-IL-6 antigen, v-IL-6 DNA, v-IL-6 RNA or antibodies against v-IL-6.
- u) A process of diagnosing the HHV-8 associated disorders kaposi sarcoma, Castleman's disease or body cavity based lymphomas (BCBL) through the diagnosis of an HHV-8 infection as described above.
- v) A process of growing cells in culture, characterized in that v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2, or mutants and variants, or fragments of v-IL-6 or the aforementioned polypeptide, or mixtures of these compounds are contained in the growth medium. In a preferred process these cells are B-lymphocytes, hybridomas, hemopoietic cells or endothelial cells.

The sequence shown in fig.2 was generated by first subcloning shotgun fragments of lambda clone G16 into commercially available plasmid pBS KS- (Stratagene, San Diego, california). Resulting plasmids were purified using a commercially available kit (Qiagen, Hilden, Germany) and sequenced on an automated sequencing system (A377, Applied Biosystems GmbH, Weiterstadt, Germany) using the recommendations of the manufacturer. The sequence was determined on both strands, using standard primers for shotgun clones, and gene specific primers for further analysis. In addition to showing the coding sequence of the interleukin-6 homologue of human herpesvirus 8, the deduced amino acid sequence, in one and three letter code, is shown in the sequence listing below.

The present invention is further described in the claims.

Bibliography:

1. Miles, S. A. et al.: *Science*, 255, 1432-1434 (1992).
2. Stürzl, M. et al.: *Oncogene* 10, 2007-2016 (1995).
3. Miles, S. A. et al.: *Proc. Natl. Acad. Sci. U. S. A.* 8
4. Masood, R. et al.: *AIDS Res. Hum. Retroviruses* 1
5. Chang, Y. et al.: *Science*. 266, 1865-1869 (1994)
6. Moore, P. S. et al.: *J. Virol.* 70, 549-558 (1996). $\leftarrow \checkmark$
7. Hammacher, A. et al.: *Protein Sci.* 3, 2280-2293
8. Ehlers, M. et al.: *J. Immunol.* 153, 1744-1753 (1994).
9. Ehlers, M. et al.: *Ann. N.Y. Acad. Sci.* 762, 400-402 (1995).
10. Albrecht, J. C. et al.: *J. Virol.* 66, 5047-5058 (1992).

Legends:**Figure 1:**

Alignment of the sequences of the predicted protein precursor of the HHV-8 IL-6 gene (SEQ ID NO: 2) with human and mouse IL-6. Amino acids identical in all three proteins are indicated by an asterisk, cysteine residues involved in disulfide bridging are marked with an arrowhead. Upper case letters symbolize amino acids conserved according to the criteria defined by M. Dayhoff.

Figure 2:

Nucleic acid sequence encoding v-IL-6 and corresponding amino acid sequence.